mide resistance in medulloblastoma. Cancer Res 32:5373–5378, 1992.
14. Bligh EG, Dyer WJ: A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917, 1959.
15. Kates M: Techniques of lipidology. Isolation, analysis and identification of lipids. In Work TS Work E (eds): "Laboratory Techniques in Biochemistry and Molecular Biology." 2nd Ed. New York: American Elsevier, pp 113–115, 1986.
16. Ellman GL: Tissue sulfhydryl groups. Arch Biochem Biophys 82:70–77, 1959.
17. Habig WH, Pabst MJ, Jakoby WB: Glutathione S-transferases—the first enzymatic step in mercapturic acid formation. J Biol Chem 249:7130–7139, 1974.
18. Nakahara I, Kikuchi H, Taki W, et al.: Changes in major phospholipids of mitochondria during postischemic reperfusion in rat brain. J Neurosurg 76:244–250, 1992.
19. Prasad MR, Popescu LM, Moraru II, et al.: Role of phospholipases A_2 and C in myocardial ischemic reperfusion injury. Am J Physiol 260:H877–H883, 1991.
20. Lin TN, Liu TH, Xu J, et al.: Brain polyphosphoinositide metabolism during focal ischemia in rat cortex. Stroke 22:495–498, 1991.
21. Chien KR, Reeves JP, Buja LM, et al.: Phospholipid alterations in canine ischemic myocardium. Circ Res 48:711–719, 1981.
22. Otani H, Prasad MR, Jones RM, Das DK: Mechanism of membrane phospholipid degradation in ischemic-reperfused rat hearts. Am J Physiol 257:H252–H258, 1989.
23. Wang LQ, Persson BG, Bergqvist L, Bengmark S: Rearterialization of a liver tumor after various dearterialization procedures. J Surg Res 57:454–459, 1994.
24. Baureisen E, Lutz J: Blood circulation and oxygen uptake in liver. Z Gastroenterol 13:70–76, 1975.
25. Granger DN, Rutili G, McCord JM: Role of superoxide radicals in intestinal ischemia. Gastroenterology 81:22–29, 1981.
26. Jaeschke H, Smith CV, Mitchell JR: Hypoxic damage generates reactive oxygen species in isolated perfused rat liver. Biochem Biophys Res Commun 150:568–574, 1988.
27. Jaeschke H, Mitchell JR: Mitochondria and xanthine oxidase both generate reactive oxygen species in isolated perfused rat liver after hypoxic injury. Biochem Biophys Res Commun 160:140–147, 1989.
28. Arroyo CM, Kramer JH, Dickens BF, Weglicki WB: Identification of free radicals in myocardial ischemia/reperfusion by spin trapping with DMPO. FEBS Lett 221:101–104, 1987.
29. Adams JD, Lauterburg BH, Mitchell JR. Plasma glutathione and glutathione disulfide in the rat: Regulation and response to oxidative stress. J Pharmacol Exp Ther 227:749–754, 1983.
30. Ross D, Cotgreave I, Moldeus P: The interaction of reduced glutathione with active oxygen species generated by xanthine-catalyzed metabolism of xanthine. Biochim Biophys Acta 841:278–282, 1985.
31. Mckelvey TG, Höllwarth ME, Granger DN, et al.: Mechanisms of conversion of xanthine dehydrogenase to xanthine oxidase in ischemic rat liver and kidney. Am J Physiol 254:G753–G760, 1988.
32. Marubayashi S, Dohi K, Ochi K, Kawasaki T: Role of free radicals in ischemic rat liver cell injury: prevention of damage by α-tocopherol administration. Surgery 99:184–191, 1986.
33. Liu X, Prasad MR, Engelman RM, et al.: Role of iron on membrane phospholipid breakdown in ischemic-reperfused rat heart. Am J Physiol, 259:H1101–H1107, 1990.
34. Terradez P, Asensi M, Lasso De La Vega MC, et al.: Depletion of tumor glutathione in vivo by buthionine sulphoximine: Modulation by the rate of cellular proliferation and inhibition of cancer growth. Biochem J 292:477–483, 1993.
35. Colvin OM, Friedman HS, Gamcsik MP, et al.: Role of glutathione in cellular resistance to alkylating agents. Adv Enzyme Regul 33:19–26, 1993.
36. Dulik DM, Fenselau C, Hilton J: Characterization of melphalan-glutathione adducts whose formation is catalyzed by glutathione S-transferases. Biochem Pharmacol 35:3405–3409, 1986.
37. Robson CN, Lewis AD, Wolf CR, et al.: Reduced levels of drug-induced DNA cross-linking in nitrogen mustard-resistant Chinese hamster ovary cells expressing elevated glutathione S-transferase activity. Cancer Res 47:6022–6027, 1987.

COMMENTARY

M. Satya Murthy, PhD†: Perhaps the authors could let us know how soon after ischemia a change in tumor size is noticeable.

Dr. Wang: We have no idea how long after ischemia a change in tumor size can be observed. Nevertheless, we do know that tumor regression cannot be induced by a single occasion of ischemia. Repeated ischemia is needed to induce a change in tumor size. Tumor size changed after the last ischemia, even checked immediately after the last treatment, because it is the result of serial inductions of ischemia and not of the last induction.

Dr. Murthy: Please explain why, in Figure 2A,B, the values in the dearterialization group are higher than in the controls.

Dr. Wang: High release of ASAT and ALAT was only found in one rat subjected to repeated dearterialization. The rest were all normal and the medians of those two groups are almost at the same level. As far as statistical significance is concerned, no difference exists between the two groups.

Dr. Murthy: How do you define irreversible damage? How does reperfusion injury add to the already irreversible damage?

Dr. Wang: Irreversible damage induced by ischemia means that cells are so severely injured that it will not recover, even if blood flow is restored, but continue to degenerate and eventually become necrotic. Reperfusion injury is recommended since it is found that much of the ischemic injury occur not during ischemia but during reperfusion. However, reperfusion injury is not an isolated event independent of ischemia but is dependent on the period of ischemia. Reactive oxygen species (ROS) is believed to be the initiator of reperfusion injury. Xanthine oxidase (XO) is an important source of superoxide in reperfused tissue. Under normal conditions, xanthine-utilizing enzymes exist predominantly as xanthine dehydrogenase (XD), which use NAD^+, rather than oxygen, as an electron acceptor and produce NADH rather than superoxide. During prolonged ischemia, proteolytic conversion of XD to XO might be triggered by an elevation of cytosolic Ca^{2+} concomitant with hypoxanthine accumulation resulting from ATP degradation. On reintroduction of oxygen, the hypoxanthine is oxidized by XO producing an excessive amount of superoxide anion and hydrogen peroxide. A severe hepatic ischemia is a prerequisite for a significant intracellular formation of ROS during reperfusion. Less than $1\frac{1}{2}$ hr of ischemia does not induce any conversion of XD to XO in the rat liver. Thus, reperfusion injury is clearly a damage originating from prolonged ischemia and revealed during reoxygenation. The irreversible injury here is induced by ischemia and part of its shows as reperfusion injury.

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